Developmental patterns of copper bioaccumulation in a marine fish model *Oryzias melastigma*

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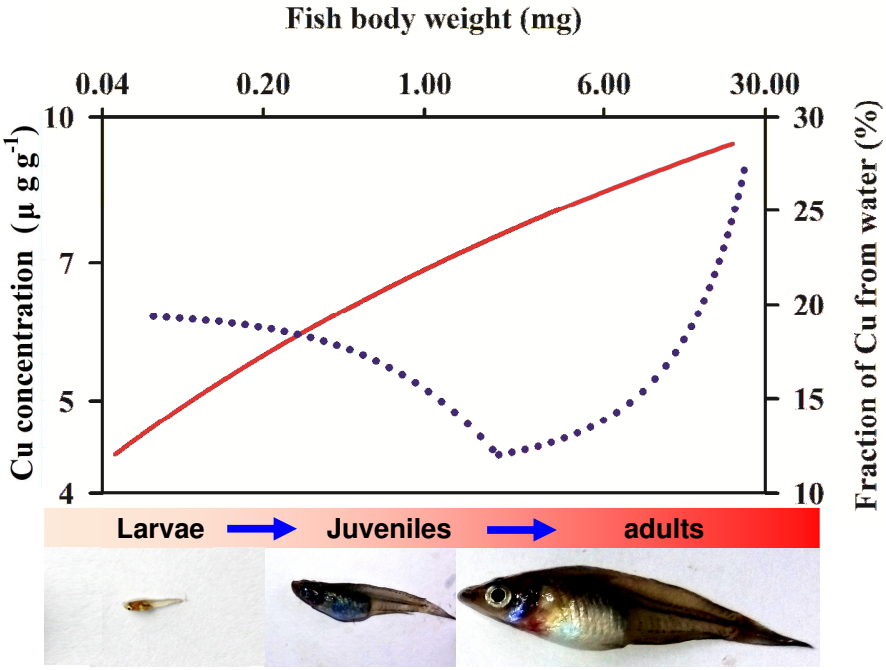
ABSTRACT

Allometry is known to be an important factor influencing metal bioaccumulation in animals. However, it is not clear whether effects are due to body size *per se* or changes in physiological traits during the animals' development. We therefore investigated the biokinetics of copper (Cu) and predicted Cu bioaccumulation during the development of a fish model, the marine medaka. The results revealed that the waterborne Cu uptake rate constant decreased and dietary Cu assimilation efficiency increased during development from larvae to adults. Thus, the allometric dependency of the biokinetic parameters in juveniles and adults can't be simply extrapolated to the whole life cycle. The body Cu concentration in the fish was predicted by the biokinetic model, which showed a rapid increase in the larval stage, followed by a slight increase from juveniles to adults, and then a relatively stable plateau in the post-adult stage. Dietary Cu uptake became more important as fish developed from larvae to juveniles, but became less important from juveniles to adults. These findings suggested that the developmental patterns of metal bioaccumulation are driven by an integrated biological/physiological shift through animals' ontogeny rather than a simple allometric dependent change. The developmental changes of metal uptake should be considered in ecological bioassessment and biomonitoring programs.

Key words: biokinetic model, ontogenetic development, metal uptake, fish, copper

Graphic Abstract



Highlights

- Cu concentration in fish shows a rapid increase in the early life stage
- Cu concentration in fish shows a stable plateau at the post-adult stage
- The importance of dietary Cu to gross body Cu concentration maximizes in medium-sized juveniles
- Biological/physiological shifts drive developmental changes in metal bioaccumulation

1. Introduction

The life of an organism usually consists of a sequence of ontogenetic steps, each with a significant shift in biological and physiological traits. The variations of these traits may have great significance in determining the bioaccumulation (Hare, 1992; Luoma and Rainbow, 2005) and toxicity of contaminants (Grosell et al., 2007; Weltje et al., 2013). In aquatic animals, for instance, the bioaccumulation of metals is often highly complicated by ontogeny, including size changes (Zhong et al., 2013; Poteat et al., 2014), growth (Zhang and Wang, 2007; Ward et al., 2010), reproduction (Conley et al., 2014), diet quantity (Guo et al., 2015a; b) or quality (Kraemer et al., 2012; Ponton and Hare et al., 2015), other physiological factors (e.g. pigment contents in insects (Hare, 1992) and condition factor (Mubiana et al., 2006; McIntyre et al., 2007)).

The body size/weight of animals, for instance, often changes greatly during the ontogeny, and it is the most frequent biological factor taken into account when investigating metal uptake in aquatic taxa (e.g. insects (Hare et al., 1992), bivalves (Wang and Fisher, 1997; Mubiana et al., 2006), and fish (McIntyre and Beauchamp, 2007; Zhang and Wang, 2007)). There is often a negative correlation between waterborne metal uptake rate and body size in aquatic animals such as insects (Hare et al., 1992), amphipods (Wang and Zauke, 2004), bivalves (Wang and Fisher, 1997) as well as fish (Newman and Mitz, 1988; Zhang and Wang, 2007; Dang and Wang, 2012; Zhong et al., 2013). Dietary metal assimilation (AEs) is also found to be size-related in some cases. For example, Zhang and Wang (2007) found AEs of the essential metals Se and Zn were positively correlated with fish body size, but the AEs of non-essential Cd was found to be independent of body size in their study. Body size might also influence the metal efflux rate (e.g. a decrease in metal efflux rate with increasing body size; Newman and Mitz, 1988; Zhao et al., 2009), although metal efflux was suggested to be relatively stable within species (Wang and Fisher, 1997). Moreover, numerous attempts have been made to illustrate the age- and size-specific patterns of heavy metal bioaccumulation in natural

129 environments, and the findings generally suggest that species- and metal-specific
130 size-related metal bioaccumulation are due to the variations in field conditions (Mubiana
131 et al., 2006; McIntyre et al., 2007; Ward et al., 2010)).

132 However, in previous studies the effects of body size (or body weight) on metal
133 bioaccumulation were often treated as a "black box". Consequently, it was not clear
134 whether the effects were due to body size *per se*, or due to shifts in other
135 biological/physiological traits during the animals' growth and development. The key
136 biological/physiological shifts, however, are often saltatory during development (e.g. the
137 changes from endogenous feeding to exogenous feeding, becoming mature and spawning
138 activities (Belanger et al., 2010)), and cannot be fully interpreted by allometry. Given this
139 fact, the allometric dependency of metal bioaccumulation may be valid within a given
140 ontogenetic stage (e.g. juveniles; Zhang and Wang, 2007; Dang and Wang, 2012) when
141 animals' biological/physiological status is relatively stable, and this yields a limited
142 picture of metal bioaccumulation for an animals' whole life cycle. For instance, it is
143 uncertain whether the allometric dependency from a single ontogenetic stage could be
144 extrapolated to the whole life cycle, and could fully describe the developmental changes
145 in metal bioaccumulation through the animals' life cycle. Therefore, further
146 understanding of the developmental dynamics of metal uptake is critical in predicting the
147 bioaccumulation and toxicity of trace metals in aquatic organisms.

148 Many fish species have been used as models in ecotoxicology research,
149 environmental risk assessment and bioassay (Van der Oost et al., 2003). A single
150 surrogate development stage is pervasively used to represent the entire life history of the
151 tested fish species (Van der Oost et al., 2003; Belanger et al., 2010). Fish, however, often
152 display a wide range of developmental ontogeny and have distinctive ontogenetic stages
153 (egg, embryo, larva, juvenile, adult, and senescence) in their life cycles. The range of
154 body sizes between conspecific individuals can usually span several orders of magnitude
155 during the development (Van der Oost et al., 2003; Belanger et al., 2010). Consequently,

156 fish size/weight is a key factor influencing metal bioavailability and bioaccumulation in
157 both laboratory (Zhang and Wang, 2007; Dang and Wang, 2012), and field studies (Ward
158 et al., 2010; Kraemer et al., 2012; Ponton and Hare et al., 2015). Nevertheless, the
159 size-related bioaccumulation of trace metals is often species-, metal- and/or site- specific,
160 suggesting weight/size is not a good predictor for metal bioaccumulation during fish
161 development (Ponton and Hare, 2015).

162 Biokinetic modelling has been applied to explore the mechanisms underlying the
163 observed size-specific bioaccumulation of metals. It has shown that body size could drive
164 the differences in metal biokinetic parameters, including dissolved metal uptake (e.g.
165 Zhong et al., 2013; Wang and Fisher, 1997), dietary metal assimilation (e.g. Zhang and
166 Wang, 2007; Dang and Wang, 2012), and metal efflux rate (e.g. Newman and Mitz, 1988;
167 Zhang and Wang, 2007). For fish species, however, few studies have focused on the
168 allometric dependence of metal biokinetic parameters in the juvenile stage (Zhang and
169 Wang, 2007; Zhong et al., 2013).These studies, at least, imply that the biokinetic model is
170 a useful tool in predicting metal bioaccumulation during fish development (Wang and
171 Rainbow, 2008).

172 Recently, the marine medaka (*Oryzias melastigma*) has been strongly proposed as a
173 new model fish for marine and estuarine ecotoxicology studies (Kong et al., 2008; Bo et
174 al., 2011), yet a few studies have investigated the ecotoxicology of trace metals on this
175 species (Wang et al., 2013). In the present study, therefore, the stable isotope ^{65}Cu was
176 used as a tracer (Croteau et al., 2004) to quantify Cu uptake biokinetics in this species at
177 three distinctive developmental stages (larvae, juveniles and adults) with the general aim
178 to explore the developmental patterns of trace metal bioaccumulation in fish. Specifically,
179 the waterborne Cu uptake, dietary Cu assimilation and body Cu efflux were quantified
180 and compared among the three developmental stages. Furthermore, we evaluated the
181 extrapolation of the allometric dependency in juveniles and adults to the whole life cycle
182 since most previous study focused on the allometric dependency of metal

bioaccumulation at juvenile and/or adult stage of fish. Finally, the developmental changes in Cu bioaccumulation, and the relative importance of Cu from waterborne vs dietary sources were predicted. Such information contributes to the understanding of the developmental changes in trace metal bioaccumulation and toxicity in fish, and has significance in environmental risk assessment and bioassay of trace metal contamination.

2. Methods and materials

2.1. Experimental fish and metals

The marine medaka has been cultured in our laboratory for more than 2 years, which represents 4 generations. The fish used in this study were at 3 development stages, including larvae (10 days post-hatching (dph)), juveniles (40 dph), and adults (120 dph)).The individual body weight (BW) was 0.15 ± 0.01, 1.34 ± 0.17, 13.32 ± 2.3 and 14.74 ± 2.01 mg (mean ± SD) in dry weight (dw) for the larvae, juveniles, adults males and adult females, respectively. The initial Cu concentrations in larvae, juveniles, males and females were 4.97 ± 0.89, 7.18 ± 1.02, 8.55 ± 1.37, 8.94 ± 1.01 μg g^{-1} in dw, respectively. The fish were housed in indoor aquaria with seawater (30 psu) at 25 ± 1 °C. The Cu concentration of the seawater was 0.98 ± 0.11 μg L^{-1}. All the fish were fed with rotifers (*Brachionus plicatilis*), the Cu content of *which* was 27.07 ± 3.24 μg g^{-1} in dw.

The stable isotope ^{65}Cu (99.8%，International Atomic Energy Agency Office at USA, New York) was used as the tracer to measure Cu biokinetic parameters. The other Cu source used was $CuSO_4 \cdot 5H_2O$ (Sigma-Aldrich), which contained Cu with typical isotopic ratios.

2.2. Uptake of waterborne Cu

There were two experiments studying uptake of waterborne Cu. In the first experiment,

208 the fish were exposed to 25 μg L^{-1} (0.38 μM L^{-1}) ^{65}Cu for 12 h and the fish were sampled
209 at 2, 4, 8 and 12 h to determine the waterborne Cu uptake rate. In the second experiment,
210 the fish were exposed to waterborne Cu with 6 concentrations, namely 5, 10, 30, 100, 200
211 and 300 μg L^{-1} (0.077, 0.15, 0.46, 1.54, 3.07, 4.69 μM L^{-1}). The first 2 solutions were
212 made entirely with ^{65}Cu, whilst the remaining 4 solutions were made using 10 μg L^{-1}
213 ^{65}Cu plus typical Cu as $CuSO_4 \cdot 5H_2O$ to bring the Cu content up to the required
214 concentration. Exposure to each solution was 4 h and the fish were sampled at 4 h to
215 determine the waterborne Cu uptake rate constant. The fish were washed using deionized
216 water for 15 minutes after sampling, which in a pilot study removed 81.4 ± 7.6 %, 84.7 ±
217 5.5 %, and 85.9 ± 8.4 % of surface sorbed Cu in larvae, juveniles and adults, respectively.
218 The seawater was filtered using 0.22 μm polycarbonate membranes (Whatman, GE
219 Healthcare). The decrease of Cu and ^{65}Cu concentrations in the seawater was less than
220 5% during the experiment. A total of 50, 10, 2 and 2 individuals were pooled as one
221 replicate for larvae, juveniles, adult males and females, respectively and each group was
222 replicated 6 times

223

224 ***2.3. Assimilation of dietary Cu***

225 Cu assimilation efficiency (AE) was measured in fish feeding on rotifers, which were
226 labeled with ^{65}Cu in the seawater containing 100 μg L^{-1} (1.54 μM L^{-1}) ^{65}Cu for 48 h. The
227 ^{65}Cu concentration of the labeled rotifers was 27.07 ± 2.13 μg g^{-1} (0.42 ± 0.03 μM g^{-1})
228 dw. Fish were maintained individually in 200 ml beakers, and there were 8 replicates for
229 each sampling time. The fish in each beaker were fed a known number of rotifers to
230 satiation over 1h and then the fish were transferred to another beaker filled with clean
231 water for depuration. The uneaten rotifers in each beaker were subsequently quantified to
232 determine the ingestion rate. There was no detectable leak of ^{65}Cu from the labeled
233 rotifers into the water during feeding. The fish were then sampled at 0, 4, 12, 24, 36, 48

234 and 60 h. During the depuration period, fish were fed with the non-labeled rotifers twice a
235 day. Feces were removed frequently and the seawater in each beaker was renewed at 4,
236 12, 24, 36 and 48 h. The ^{65}Cu concentration in seawater did not elevate significantly
237 during depuration (1.08 ± 0.17 μg L^{-1} and 1.16 ± 0.21 μg L^{-1} at the start and end of the
238 depuration time, respectively).

239

240 ***2.4. Cu efflux***

241 The fish were exposed to 20 μg L^{-1} (0.31 μM L^{-1}) ^{65}Cu spiked seawater for 7 d to
242 accumulate ^{65}Cu, and were then transferred to non-spiked seawater for a 28 d depuration
243 period. During the depuration, the fish were sampled at 1, 2, 4, 8, 12, 16, 20, 24, and 28 d.
244 The fish were fed twice a day and the seawater renewed daily.

245

246 ***2.5. Cu and ^{65}Cu content analysis***

247 All fish and rotifer samples were oven-dried at 60 °C for 48 h, and digested using HNO_3
248 (70%, ultrapure, Fisher Scientific, Geel, Belgium) for 48 h at 80 °C. The seawater
249 samples were digested using HNO_3 for 48 h at ambient temperature. The digested
250 samples were then diluted with 2% nitric acid. The total Cu and ^{65}Cu contents in all
251 samples were quantified by inductively coupled plasma-mass spectroscopy (7700X,
252 Agilent Technologies Inc., California, USA). An appropriate internal standard (i.e. ^{72}Ge)
253 was selected to correct the sensitivity drift and matrix effects. A quality control sample
254 was analysed every 20 samples. Recalibration was conducted if the recovery of Cu from
255 the quality control deviated by more than ± 10% from the correct concentration. The
256 efficacy of analysis methods were evaluated by the analysis of a tuna fish standard
257 reference material (BCR-627, Institute for Reference Materials and Measurements, Geel,
258 Belgium). The recovery of Cu from this was 90.47 ± 5.04 %. The net increase of ^{65}Cu in
259 the samples was determined as described by Croteau et al. (2004).

260

2.6. Modeling

The biokinetic model was used to describe Cu bioaccumulation (Luoma and Rainbow, 2005; Wang and Rainbow, 2008):

$$dC_t / dt = k_u \times C_w + AE \times IR \times C_f - (k_e + g) \times C_t \quad (1)$$

where C_t is the Cu concentration at time t, k_u is the uptake rate constant of water Cu ($L\ g^{-1}\ d^{-1}$), C_w is the Cu concentration in water ($\mu g\ L^{-1}$), AE is the dietary Cu assimilation efficiency, IR is the ingestion rate ($g\ g^{-1}\ d^{-1}$), C_f is the Cu concentration in diet ($\mu g\ g^{-1}$), k_e is the efflux rate constant (d^{-1}), and g is the growth rate (d^{-1}).

The waterborne Cu uptake rate (J_w, $ng\ g^{-1}\ h^{-1}$) was calculated as the slope of the linear regression between the net increase of ^{65}Cu and exposure time. The k_u was defined according to the equation :

$$k_u = J_w / C_w^b \quad (2)$$

where b is the kinetic order.

IR was calculated as:

$$IR = W_i \times (N_f - N_u) / W_f \quad (3)$$

where W_i is the dry body weight of a single rotifer, N_f is the number of rotifers fed to the fish and N_u is the number of uneaten rotifers. W_f is the dry body weight of the fish.

The AE was calculated as:

$$AE = A_{48\text{-}60\,h} / A_{0\,h} \times 100 \quad (4)$$

where $A_{48\text{-}60\,h}$ is the ^{65}Cu retained in the whole fish at 48 and 60 h, and $A_{0\,h}$ is the initial ^{65}Cu in the whole fish (Figure 2A).

The k_e was calculated from the slope of the linear regression between the natural logarithm of the percentage of ^{65}Cu ($p^{65}Cu$) and depuration time during 4-28 d (d, Figure 3A) as:

$$k_e = \log (p^{65}Cu) / d + b$$

where b is the intercept

The g was calculated as:

$$g = \ln\ (\text{final body weight} / \text{initial body weight}) / \text{days} \quad (6)$$

The fraction of Cu accumulated from the water (f) was calculated as:

$$f = k_u / (k_u + \text{AE} \times \text{IR} \times \text{BCF}) \quad (7)$$

where BCF was the bioconcentration factor of Cu in diets.

2.7. Statistical analysis

The differences of AE among the four groups were analyzed using one-way ANOVA followed by a Tukey's HSD *post-hoc* test. Analysis of covariance (ANCOVA) was used to test the differences in J_w, k_u, and k_e among the four groups (with the body weight as the covariate). A non-linear regression was used to interpret the correlation between J_w, k_u, k_e, g, food ingestion rate and body weight of the fish. Difference was regarded as significant when $p < 0.05$. All statistical analyses were performed by SPSS (vs. 18 SPSS Inc., Chicago, USA) and SigmaPlot (vs. 12 Systat Software Inc., California, USA).

3. Results

3.1. The uptake kinetics of waterborne Cu

The J_w of Cu in the larvae was more than two-fold higher than that in the juveniles, and three-fold higher than in the adults (Table 1). The larvae had a significantly higher k_u than the other groups (Figure 1B; Table 1). From larvae to adults, the k_u and fish body weight were expressed as a negative power function ($k_u = 43.80\text{BW}^{-0.10}$), whereas the k_u did not significantly correlate with fish body weight in the juveniles or adults (Fig. 1C).

3.2. The assimilation of dietary Cu

The ingested ^{65}Cu retained in the fish was constant after depuration for 48-60 h, and the

AE for dietary Cu in the larvae was significantly lower than in the other three groups (one-way ANOVA, $p < 0.05$, Fig. 2A). Overall, AE was scaled to the fish body weight with an allometric coefficient of 0.13 (Fig. 2B). From the juvenile to adult stage, however, the allometric coefficient of AE (0.09) was lower than that from the larvae to adult stage (Fig. 2B)

3.3. The efflux of Cu

The fish rapidly lost Cu from the body in the first 4 d, and then showed a slower loss rate from 4 to 28 d (Fig. 3A). The k_e of larvae was significantly higher than that of females (Table 2). From the larva to adult stage, the k_e showed a negative correlation with fish body weight ($k_e = 0.047BW^{-0.09}$, Figure 3B), while a lower allometric coefficient of k_e (-0.12) was found from juveniles to adults (Fig. 3B).

3.4. The modeling of developmental patterns of Cu bioaccumulation

Other than the biokinetic parameters detected above, other parameters used for the modeling included the specific growth rate (g, 0.096, 0.033, 0.0079 and 0.0070 d^{-1} for larvae, juveniles, males and females, respectively, in Fig. 5A), and ingestion rate (IR, 0.34, 0.19, 0.07 and 0.08 g g^{-1} d^{-1} for larvae, juveniles, males and females, respectively, in Fig. 5B). Developmental changes in the Cu concentrations of the fish were predicted by the biokinetic model using the measured parameters (Fig. 4A). The model prediction suggested that the Cu concentrations in the fish would increase sharply in early development, followed by a low rate of increase from juveniles to adults, before then reaching a relatively stable plateau in the post-adult stage (Fig. 4A).

When calculating the fraction of Cu from water (f, %), the bioconcentration factor (BCF) of Cu in the diets was set within a range of the natural fish food, i.e. 1×10^3 to $3 \times$

10^4 L kg^{-1} (McGeer et al., 2003; USEPA, 2007; Dang et al., 2009). In eqs 7, the AE = $11.27BW^{0.13}$ (Fig. 4B), and IR = $0.19BW^{-0.28}$ (Fig. 5B). The *f* was apparently high in the larva stage and displayed a decrease when the fish developed to juveniles, while the *f* showed a clear increase from post-juveniles to adults (Fig. 4B). Moreover, *f* generally decreased greatly with increasing BCF and dietary Cu dominated the total Cu bioaccumulation at a high BCF (e.g. BCF > 10^4).

4. Discussion

4.1. The prediction of developmental patterns of metal bioaccumulation

The increase in Cu content in the fish *O. melastigma* during ontogenic development was predicted by the biokinetic model using the measured parameters (Fig. 4A). Similar relationships between metal (or metalloid) content and body size were also observed in several other studies. For instance, Ponton and Hare (2015) reported that Se concentrations in field-collected yellow perch (*Perca flavescens*) increased significantly with fish weight in the early life stage. Nevertheless, when the fish weight was above ~28 g, Se concentrations either levelled off or slightly declined. These results are consistent with our findings that the Cu concentrations in fish increased rapidly in early development and are relatively stable in the adult stage. Furthermore, Douben (1989) found that Cd concentrations in stone loach (*Noemacheilus barbatulus*) reached a steady state in fish of 2 years old or more, but not in younger fish, which is again consistent with a stable Cu concentration in the post-adult stage of the present study. Aditionally, we did not observed a clear difference in Cu concentration between males and females, which was probably due to a lack of egg laying by females during the study period (Conley et al., 2014).

4.2. The effect of development changes on the relative importance waterborne vs

364 ***dietary metal***

365 Fish in the early life-history stage accumulated a high proportion of Cu from the water.
366 This mainly resulted from a higher capacity for Cu uptake via water and/or a lower AE in
367 larvae in relation to juveniles and adults (Dang and Wang, 2012). Interestingly, when fish
368 developed from juveniles to adults, the deceasing ingestion rate was the main contributor
369 to the increasing proportion of waterborne Cu taken up, as the magnitude of the decrease
370 in k_u (decreasing by ~8% in the present study) is much less than the decrease in AE × IR
371 (decreasing by 51% in the present study). These results are the first to demonstrate that
372 the maximal proportion of Cu uptake via the diet occurred in medium sized juveniles, and
373 that dietary Cu uptake was less important for small sized larvae or large sized adults.
374 Nevertheless, it is necessary to keep in mind that the dietary Cu dominates the gross Cu
375 bioaccumulation, especially when IR and BCF are at medium/high levels (Kamunde et al.,
376 2002; Dang et al., 2009).

377

378 It is well acknowledged that small-sized fish in early life stages are much more sensitive
379 to waterborne Cu compared to larger ones, which is mainly attributed to variations in
380 size-dependence of physiological tolerances among the life stages (Grosell et al., 2007;
381 Weltje et al., 2013). The results of the present study suggest an alternative mechanism,
382 that the high sensitivity of larvae to waterborne Cu could also be attributed to a high
383 waterborne Cu uptake rate (Niyogi and Wood, 2004; USEPA, 2007; Weltje et al., 2013).
384 The findings further suggested that the fish in the larvae stage (e.g. < 1 mg, Figure 4B)
385 might be exclusively applicable for the biomonitoring of waterborne metals due to their
386 high waterborne uptake rate and the higher proportion of metal deriving from the water,
387 whereas the juveniles (e.g. ~1-~5 mg) ought to be more sensitive to the metal
388 contamination in diets as they have a high AE and accumulate a high proportion of metal
389 from the diet. These findings substantially improve the theoretical understanding for the
390 validity and applicability of this fish model in ecological bioassessment and

391 biomonitoring programs.

392

393 ***4.3. Allometric dependency can't fully interpret the developmental patterns of metal***
394 ***bioaccumulation***

395 There were clear developmental changes in the key biokinetic parameters for Cu uptake,
396 including k_u, IR, AE, k_e and g, which drive the developmental patterns of metal
397 bioaccumulation in the fish (Zhang and Wang, 2007; Dang and Wang, 2012). The ratio of
398 body surface area to body weight has a constant of -0.67 (-2/3). The allometric exponent
399 of these parameters is not close to -0.67 (i.e. the ratio of body surface to volume),
400 suggesting developmental variations of the biokinetic model parameters can't be solely
401 interpreted in terms of morphological characteristics during fish ontogeny (Canli and Atli,
402 2003; Dang and Wang, 2012). For instance, an allometric exponent of k_u close to -0.67
403 suggests the waterborne metal uptake is a highly allometric dependent process and vice
404 versa. The allometric exponent of k_u varies among species and metals, and it is often not
405 close to -0.67 (e.g. Newman and Mitz, 1988; Wang and Zauke, 2004; Pan and Wang,
406 2008; Chen et al., 2014).

407 The *ku* is mainly determined by the number and efficiency of biotic metal binding sites
408 on organisms' body, suggesting it should be allometric dependent (Niyogi and Wood,
409 2004). Whilst it is also influenced by several physiological processes, such as
410 osmoregulation (USEPA, 2007) and ventilation rate (Wood et al., 2010). Similarly, the *ke*
411 decreases with the animals' size as small-sized animals have a high metabolic rate scaled
412 to body mass, which might increase metal turnover and equilibrium (Newman and
413 Mitz,1988; Zhao et al., 2009), and thus lead to a high metal efflux (Baines and Fisher,
414 2008). Collectively, our findings suggested that the bio-logical/physiological
415 characteristics rather than a sole allometric change result in the size-specific metal
416 bioaccumulation seen in both natural environments (e.g. Canli and Atli, 2003; Mubiana et

417 al.,2006; McIntyre and Beauchamp, 2007) and laboratory situations (e.g. Wang and
418 Fisher, 1997a,b; Zhang and Wang, 2007; Pan and Wang, 2008).

419 Additionally, our results suggested that the allometric dependency of the biokinetic
420 parameters (e.g. k_u, AE, k_e) based on juveniles and adults can't be simply extrapolated to
421 the whole life cycle, mainly owing to the saltatory changes in ontogenic stage, which has
422 been ignored in previous studies. AE, for instance, showed a sharp increase from larvae to
423 juveniles, owing to the development of digestive organs and gastrointestinal tract (Infante
424 and Cahu, 2007), and/or the high IR in larvae in relation to juveniles and adults (Zhang
425 and Wang, 2006; Guo et al., 2015). A significantly higher k_u and k_e in the larvae was
426 observed as well. Thus, the biokinetic parameters at each key ontogenetic step should be
427 quantified to understand the developmental patterns of metal bioaccumulation in aquatic
428 animals.

429 Overall, this study predicted the developmental changes of Cu bioaccumulation in a
430 new marine fish model, and addressed the effect of development changes on the relative
431 importance of waterborne vs dietary metal. The prediction has a wide generality and
432 applicability for most fish species as the saltatory ontogeny of physiological traits in fish
433 are easily identified and show generality as well. Theoretically, the framework of the
434 prediction is flexible and applicable to other aquatic organisms with further knowledge
435 on developmental changes in biokinetic parameters for metal assimilation. However, the
436 development patterns of metal accumulation in aquatic animals are highly variable in
437 natural environments given the biological/physiological characteristics, as the main
438 drives for the changes of biokinetic parameters, may be sensitive to environment factors
439 (e.g. Luoma and Rainbow, 2005; Wang and Rainbow, 2008; Poteat et al., 2013).
440 Consequently, the sensitivity and validity of the prediction to critical environment factors
441 (e.g. water temperature (Baines and Fisher, 2008), food availability (Zhao et al., 2015;
442 Guo et al., 2015)) should be tested in field situations.

443

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563

564 **Figure Legends**

565

566 **Fig. 1.** The regression of the newly bioaccumulated Cu concentrations (C) with exposure
567 time (T, panel A), the waterborne Cu uptake rate (J_w) with waterborne Cu concentration
568 (C_w, panel B), and the uptake rate constant (k_u) with the fish body weight (BW, panel C)
569 in the marine medaka. Values of each point are means ± standard deviations (n = 6 in the
570 panel A and panel B, and n= 24 in the panel C). In panel C, $k_u = 43.80BW^{-0.10}$ from the
571 larva to adult stage ($r^2 = 0.88$, $p = 0.03$), and $k_u = 36.69BW^{-0.02}$ from the juvenile to adult
572 stage ($r^2 = 0.13$, $p = 0.76$).

573

574 **Fig. 2.** The retention of ingested dietary Cu after pulse feeding of *Brachionus plicatilis*
575 (panel A), and the regression of the assimilation of dietary Cu (AEs) with fish body
576 weight (panel B) in the marine medaka. Values of each point are means ± standard
577 deviations (n = 8 in the panel A, and n= 16 in the panel B). In panel B, $AE = 11.27$
578 $BW^{0.13}$ from the larvae to adult stage ($r^2 = 0.97$, $p = 0.002$), and $AE = 11.27BW^{0.09}$ from
579 the juvenile to adult stage ($r^2 = 0.98$, $p = 0.001$).

580

581 **Fig. 3.** The retention of body Cu burden after 7 d waterborne Cu exposure (panel A), and
582 the regression of the Cu efflux rate constant (k_e, d^{-1}) with fish body weight of the marine
583 medaka (panel B). Values are mean ± standard deviation (n = 6). In the panel B, $k_e =$
584 $0.047BW^{-0.09}$ from the larva to adult stage ($r^2 = 0.84$, $p = 0.031$), and $k_e = 0.047BW^{-0.12}$
585 from the juvenile to adult stage ($r^2 = 0.64$, $p = 0.37$).

586

587 **Fig. 4.** The model-predicted developmental changes of the Cu concentrations (panel A),
588 and the fraction of Cu bioaccumulated from water (panel B) in the marine medaka. In the
589 panel A, the waterborne Cu was 0.98 μg L^{-1} and the dietary Cu was 27.07 μg g^{-1}
590 measured in this study. The solid lines are model predicted, and the dashed lines are the
591 1:1 lines, and r^2 is the coefficient of dependence of the 1:1 line between the predicted and

592 measured values (n = 64). In the panel B, the bioconcentration factor of Cu of diets (BCF)
593 was 1×10^3 (dash-dot-dot line), 5×10^3 (dash line), 1×10^4 (dotted line) and 3×10^4
594 (solid line).
595
596
597 **Fig. 5.** The correlation of the specific growth rate (panel A), and food ingestion rate of the
598 fish (panel B) with fish body weight in the marine medaka. Values of each point are
599 means ± standard deviation (n = 36 in the panel A, and n= 64 in the panel B).

Table 1 The waterborne Cu influx rate (J_w, μg g^{-1} h^{-1}) and waterborne Cu uptake rate constant (k_u, L g^{-1} h^{-1}) of larvae, juveniles, males and females of the marine medaka. The J_w was calculated from the slope of the linear regression between the newly bioaccumulated Cu concentrations (C, ng g^{-1} in dry weight) and exposure time (T, h) (Figure 1A). The k_u calculated from the regression between the Cu influx rate (J_w, μg L^{-1} d^{-1}) and waterborne Cu concentrations (C_w, μg L^{-1}) by function of $J_w = k_u \times C_w^b$ (Figure 1B). The J_w and k_u with different superscript are significantly different between the development stages (ANCOVA, $p < 0.05$). The SE (standard error), r^2 and p values derive from the regression.

Development stages	J_w				k_u				
	J_w ($\times 10^{-2}$)	SE ($\times 10^{-2}$)	r^2	p	k_u ($\times 10^{-3}$)	SE ($\times 10^{-3}$)	b	r^2	p
Larvae	7.63^{a}	0.30	0.98	0.001	56.45^{a}	3.02	1.034	0.92	<0.001
Juveniles	3.52^{b}	0.44	0.94	0.011	35.89^{b}	2.71	1.010	0.95	<0.001
Adult-Males	2.44^{bc}	0.11	0.96	0.004	34.77^{b}	4.05	1.023	0.97	0.004
Adult-Females	2.12^{c}	0.13	0.97	0.006	31.42^{b}	3.55	1.041	0.93	<0.001

Table 2 The Cu efflux rate constant (k_e, d^{-1}) calculated from slope of the linear regression between the natural logarithm of the percentage of retained ^{65}Cu and the depuration time from 4 to 28 d (Figure 2B). The k_e with different superscript are significantly different between the treatments (ANCOVA, $p < 0.05$). The SE (standard error), r^2 and p values derive from the regression.

Development stages	k_e ($\times 10^{-2}$)	SE ($\times 10^{-2}$)	r^2	p
Larvae	4.92^{a}	0.75	0.89	0.0012
Juveniles	4.23^{ab}	0.88	0.78	0.0049
Adult-Males	3.63^{ab}	0.68	0.82	0.0029
Adult-Females	3.24^{b}	0.63	0.78	0.0055

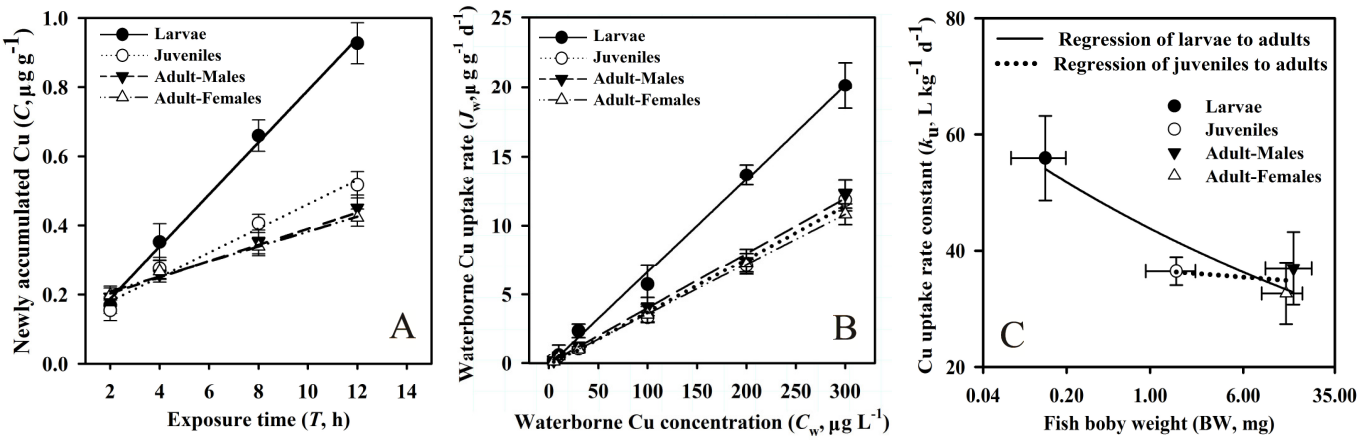
Larvae
Juveniles
Adult-Males
Adult-Females
Newly accumulated Cu (C, μ g g-1)
Exposure time (T, h)
A
Waterborne Cu uptake rate (Jw, μ g g-1 d-1)
Waterborne Cu concentration (Cw, μg L-1)
B
Regression of larvae to adults
Regression of juveniles to adults
Cu uptake rate constant (ku, L kg-1 d-1)
Fish boby weight (BW, mg)
C

Fig. 1.

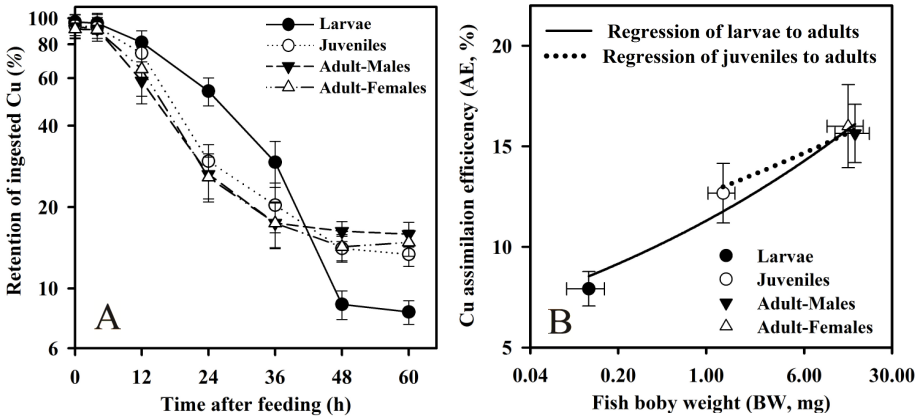
Retention of ingested Cu (%)
100
80
60
40
20
10
6
0
12
24
36
48
60
Time after feeding (h)
Larvae
Juveniles
Adult-Males
Adult-Females
A
Cu assimilaion efficicency (AE, %)
20
15
10
5
0.04
0.20
1.00
6.00
30.00
Fish boby weight (BW, mg)
Regression of larvae to adults
Regression of juveniles to adults
Larvae
Juveniles
Adult-Males
Adult-Females
B

Fig. 2.

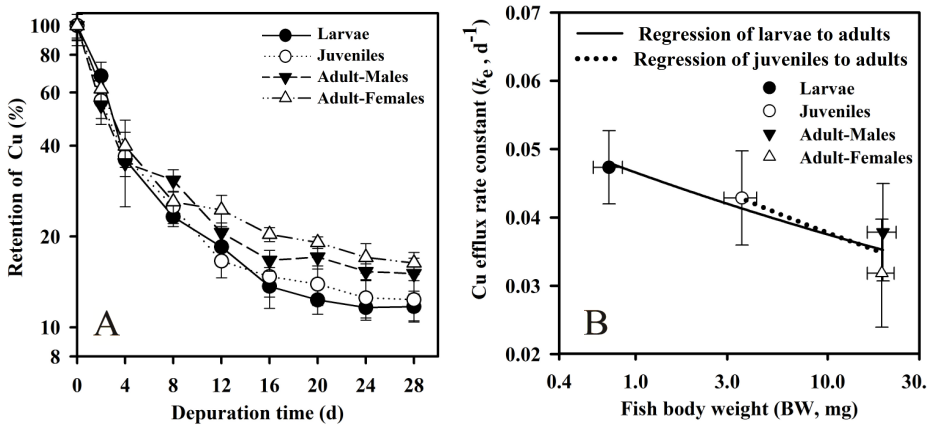
Retention of Cu (%)
100
80
60
40
20
10
8
A
0
4
8
12
16
20
24
28
Depuration time (d)
Larvae
Juveniles
Adult-Males
Adult-Females
Cu efflux rate constant (k_e, d^{-1})
0.07
0.06
0.05
0.04
0.03
0.02
B
0.4
1.0
3.0
10.0
30.
Fish body weight (BW, mg)
Regression of larvae to adults
Regression of juveniles to adults
Larvae
Juveniles
Adult-Males
Adult-Females

Fig. 3.

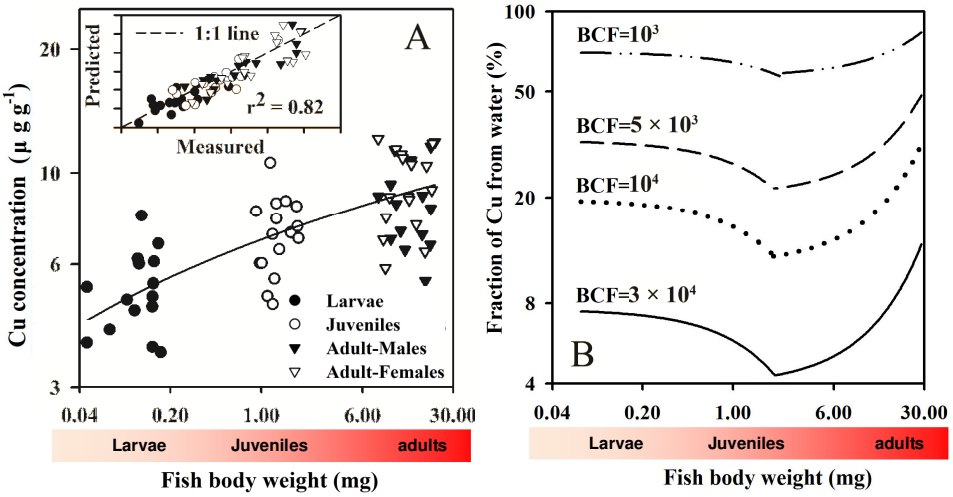
A
1:1 line
Predicted
Measured
r2 = 0.82
Cu concentration (µ g g-1)
20
10
6
3
Larvae
Juveniles
Adult-Males
Adult-Females
0.04
0.20
1.00
6.00
30.00
Larvae
Juveniles
adults
Fish body weight (mg)
B
BCF=10³
BCF=5 × 10³
BCF=10⁴
BCF=3 × 10⁴
Fraction of Cu from water (%)
100
50
20
8
4
0.04
0.20
1.00
6.00
30.00
Larvae
Juveniles
adults
Fish body weight (mg)

Fig. 4.

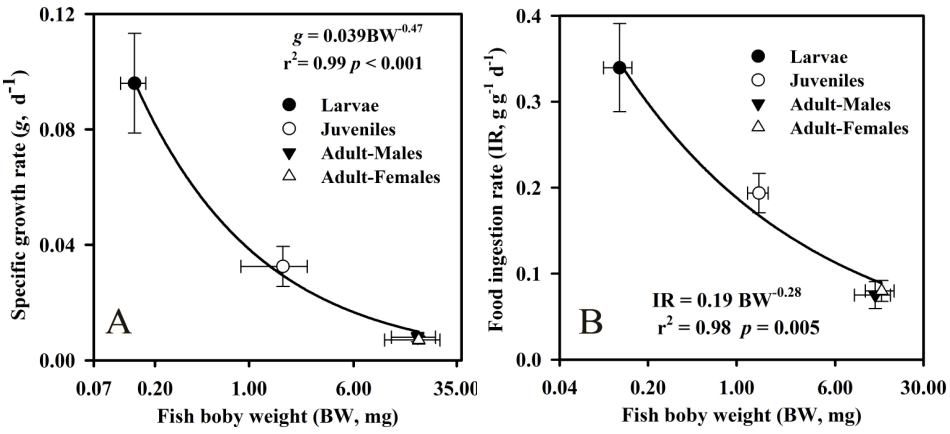
0.12
0.08
0.04
0.00
Specific growth rate (g, d-1)
g = 0.039BW-0.47
r2= 0.99 p < 0.001
Larvae
Juveniles
Adult-Males
Adult-Females
A
0.07
0.20
1.00
6.00
35.00
Fish boby weight (BW, mg)
0.4
0.3
0.2
0.1
0.0
Food ingestion rate (IR, g g-1 d-1)
Larvae
Juveniles
Adult-Males
Adult-Females
B
IR = 0.19 BW-0.28
r2 = 0.98 p = 0.005
0.04
0.20
1.00
6.00
30.00
Fish boby weight (BW, mg)

Fig. 5.